Novel treatment targets in high-grade brain tumors

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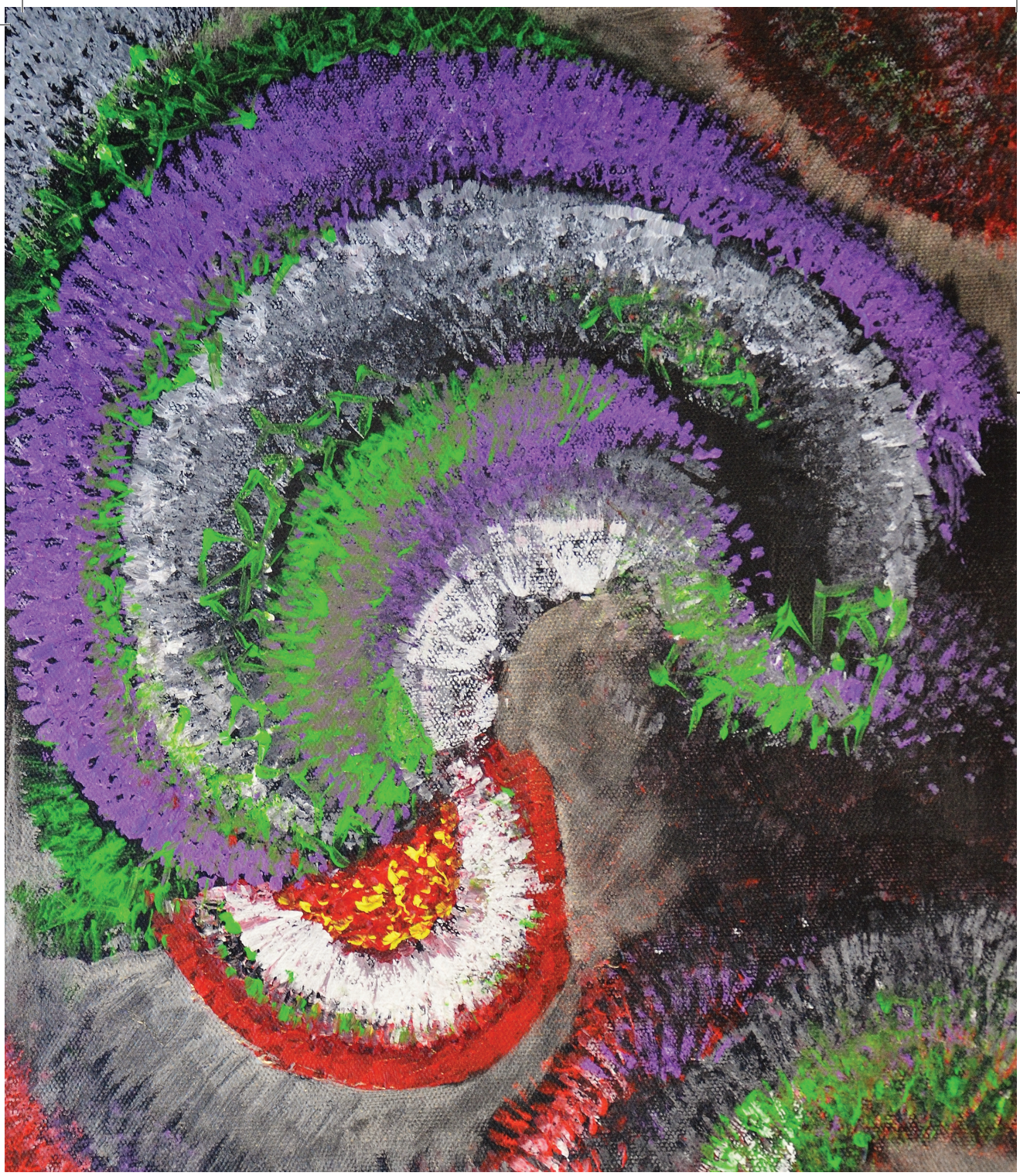

***Memories of the Ocean**: Hippocampus of the mouse brain. The hippocampus, named after its resemblance to the seahorse, is the center for short term memory. It weighs the importance of episodic acts and decides which should be kept as memories*

Chapter 8

Summary | Samenvatting

SUMMARY

In chapter 1 we provide a background on high-grade brain tumors, with a focus on the most common pediatric and adult high-grade brain tumors, the medulloblastoma (MB) and the glioblastoma (GBM), respectively. Despite the increase in our understanding of the (epi) genetic background of MB and GBM, outcome in these tumors remains poor and survivors suffer from severe late effects. The aim of this thesis was to identify novel treatment targets for the high-grade brain tumors MB and GBM. To do so we have focused on three different processes driving high-grade brain tumors: 1) developmental pathways, 2) angiogenesis, and 3) therapy resistance.

Deregulation of important developmental pathways drives the formation of MB. Recent studies show that deregulated post-translational modifications of histone H3 lysine 27 (H3K27) are common events in MB. This prompted us to investigate the trimethylation status of H3K27, as well as the expression of the histone methyltransferase EZH2 and histone demethyltransferase KDM6B, during human cerebellum development and in MB. **In chapter 2**, we provided evidence that trimethylation of H3K27 is upregulated in MB samples compared to normal cerebellum. Moreover, during development of the human cerebellum trimethylation of H3K27 and its modifiers is detected in a spatio-temporal manner, with consistent high occurrence in the four proliferative zones of the cerebellum, which are believed to harbor the precursor cells of the different MB subgroups. These results indicate that H3K27 trimethylation is deregulated in MB. Moreover, the presence of H3K27me3 and its modifiers during development identifies a subset of cerebellar precursors as putative cells of origin for the H3K27 deregulated MBs.

Angiogenesis is an important driver of tumor progression in GBM. The vascular endothelial factor (VEGF) has been shown to play a major role in GBM angiogenesis. In chapters 3 and 4 we investigate the role of epigenetic and microRNA (miRNA) signaling in the GBM angiogenesis. **In chapter 3** we demonstrate that the histone methyltransferase EZH2 is highly expressed in high-grade gliomas, including GBM, and recurrent gliomas. Furthermore, upregulation of EZH2 is partly driven by decreased expression of miR-101 in GBM. Functional *in vitro* studies provide evidence that targeting EZH2, either with siRNA or pharmacologically, or by upregulating miR-101 results in decreased proliferation, migration, invasion, and angiogenesis in GBM cell lines. Finally, using a small molecule EZH2 inhibitor we prove that decreasing EZH2 levels reduces tumor growth in an *in vivo* GBM xenograft mouse model. **In chapter 4** we focus on angiogenesis driven by EZH2 and miR-101in GBM endothelial cells. Here, we identify a VEGF/miR-101/EZH2 axis. GBM cells produce VEGF, which is shown to down regulate miR-101 in tumor-associated endothelial cells. The reduced miR-101 expression results in increased expression of EZH2 in endothelial cells, which partly causes a

pro-angiogenic effect. Finally, reducing EZH2 expression by a small molecule inhibitor results in reduced blood vessel formation in GBM xenograft mouse model.

GBMs have an inherent resistance to both chemo- and radiotherapy. Pathways causing resistance to conventional therapy are focus of chapters 5 and 6. **In chapter 5**, we review the role of the WEE1 kinase in mediating resistance to DNA-damaging agents in cancer. We describe the role of WEE1 in cell cycle control and initiation of DNA-repair after damage. Results from preclinical and clinical studies using selective WEE1 inhibitor are described. **In chapter 6**, using *in silico* analyses of kinase expression we identify the WEE1 kinase as novel target for the treatment of GBM. We demonstrate that WEE1 is overexpressed in GBM and is a major regulator of the G2 checkpoint in GBM cells. Inhibition of WEE1 by siRNA or small molecular compound in cells exposed to DNA damaging agents results in abrogation of the G2 arrest, premature termination of DNA repair, leading to mitotic catastrophe and cell death. Importantly, a small-molecule inhibitor of WEE1 sensitizes GBM to ionizing radiation *in vivo*. Our results suggest that inhibition of WEE1 kinase holds potential as a therapeutic approach in treatment of GBM.